Massachusetts Department of Public Health

State Laboratory Institute

A Monthly Newsletter of the Bureau of Laboratory Sciences

Volume 1, Number 7 July, 1999

National Conference to be Held at Massachusetts Sites

"Response to Bioterrorism - Role of the Clinical Laboratory"

As planning for "Response to Bioterrorism" is defined, a national conference will be presented by expert speakers from CDC, Public Health, FBI and the Medical Community to define the role of the clinical laboratory. Several sites in Massachusetts will host the conference:

September 9: State Laboratory Institute, Jamaica Plain, MA.

November: Pittsfield and Berkshire Regions (details to follow).

Topics for this 1-day conference include:
- Overview of Bioterrorism
- Bioterrorism Threats and Hoaxes
- Laboratory Levels and Capabilities
- What Every Laboratory Needs to Know
- Clinical Aspects of Critical Biological Agents

Clinical laboratorians, safety officers, nurses, physicians and health officials are encouraged to attend this important conference. For a brochure and more information call 800-536-NLTN or 617-983-6285 or visit the web site at www.phppo.cdc.gov/dls/nltn.

This program is sponsored by the National Laboratory Training Network (NLTN), the Centers for Disease Control and Prevention (CDC), and the Association of Public Health Laboratories (APHL).

Pediatric Blood Lead Testing

by Julianne Nassif

Massachusetts requires blood lead screening at least annually for all children under the age of 48 months beginning at 9-12 months of age. Children who are at high risk for lead poisoning, e.g., living in older housing that is in poor condition or having a sibling who is lead poisoned, are tested at an earlier age and on a more frequent basis. Prior to entrance into kindergarten, all children must have proof of blood lead screening.

The State Laboratory Institute tests more than 150,000 specimens per year, or about 50% of all specimens analyzed in Massachusetts. Fingerstick and venipuncture specimens collected by pediatric healthcare providers are sent to the laboratory by courier or mail. Nearly all specimens received are analyzed on the day received (>98%) and specimens received at the end of the day are analyzed and reported the following day. Patient reports are mailed to health care providers on a daily basis. In addition, blood lead (PbB) data are sent electronically to public health nurses working in the state and City of Boston offices for Childhood Lead Poisoning Prevention.

Lead concentration in whole blood specimens is determined by graphite furnace atomic absorption spectroscopy (GFAAS) with Zeeman background correction following dilution with matrix modifiers. The sample is thermally decomposed and elemental lead is atomized. The gas is exposed to a light source at 283 nanometers, the wavelength characteristic of lead absorption, and absorbance is measured. The measured absorbance is directly proportional to the concentration of lead in the specimen.

Educational services are offered to children with blood lead levels of 10 μg/dL or greater.

Samples with lead concentrations equal to or greater than 15 μg/dL are retested to confirm the measurement. Specimens with an elevated PbB are also tested for free erythrocyte protoporphyrin, a precursor of hemoglobin. This additional information regarding the child's iron status is often useful for clinical management of the child.

Children with PbB of 20 μg/dL and greater are enrolled in clinical case management with the Massachusetts CLPPP. This entitles the child and family to support services, including a free home lead-based paint inspection, exposure reduction education, and coordination of clinical management of the child's care by a public health nurse.

In addition to analyzing specimens, the laboratory maintains a comprehensive patient based data system for CLPPP of pediatric blood lead testing that includes test results from both SLI and private laboratories, which are required by regulation to report all test results to the Department of Public Health.

In This Issue

Grants, Projects & Publications

Characterization of *Bordetella holmesii* Recovered from Respiratory Specimens of Patients with Pertussis-Like Symptoms[1]

E. Mazengia, H. George, E. Silva and J. Peppe

From January 1995 through December 1998, the Massachusetts State Laboratory Institute (SLI) isolated *B. holmesii* from 32 nasopharyngeal specimens of patients with pertussis-like symptoms. Previously, *B. holmesii* had been associated with septicemia in patients with a variety of underlying conditions. Therefore, a study was undertaken to describe the recovery of *B. holmesii* from nasopharyngeal specimens and also to report on the use of Pulsed Field Gel Electrophoresis (PFGE) as an epidemiological tool for determining genotypic relatedness among cases of *B. holmesii*.

Thirty-two *B. holmesii* clinical isolates and 14 isolates representing three other *Bordetella spp.*, *B. bronchiseptica* (four isolates), *B. parapertussis* (five isolates) and *B. pertussis* (five isolates), were included in the study. Each of the isolates was inoculated on Bordet Gengou media (BG) with the following antibiotic variations: 0.625 μg/mL oxacillin, 40 μg/mL cephalexin, 2.5 μg/mL methicillin and no antibiotic (control). In addition, each was inoculated on Charcoal Agar (CA) with 40 μg/mL of cephalexin and without antibiotic. In our laboratory CA with cephalexin is used as the transport medium, and BG with methicillin is used as a secondary culture medium. We also investigated the genotypic relatedness of the recovered *B. holmesii* isolates using PFGE. The DNA from each of the *B. holmesii* isolates was prepared and restricted with *XbaI* and *SpeI* for Pulsed Field Gel Electrophoresis.

We found that cephalexin, the antibiotic commonly incorporated in both CA and the BG, is inhibitory to the growth of *B. holmesii*. However, all of the *Bordetella spp.* were resistant to methicillin and oxacillin. Therefore, the recovery of *B. holmesii* can be achieved by the substitution of either methicillin or oxacillin for cephalexin in CA and BG media.

The genotypic analysis of the 32 *B. holmsii* isolates by PFGE following restriction with *XbaI* identified 5 distinct patterns, whereas, restriction with *SpeI* resulted in only 3 patterns. The PFGE provided supporting evidence that there were no linkages between the various cases of *B. holmesii* and identified the dominant circulating strain during the study period. For information concerning specific conditions that were used for PFGE, contact Eyob Mazengia at (617) 983-6612 or eyob.mazengia@state.ma.us.

[1] The original abstract was presented at the General Meeting of the American Society for Microbiology, May 30-June 3, 1999.
E. Mazengia is a fellow at the Massachusetts State Laboratory Institute under the APHL/CDC Emerging Infectious Diseases Laboratory Fellowship Program.

Quality Assurance & Regulations

Method Verification for FDA Approved Test Methods

by Dina Caloggero

Laboratories performing high-complexity testing must verify specific performance characteristics according to CLIA '88 and CAP regulations before reporting patient test results. There are two categories for verification requirements. The first category includes FDA approved test methods, which are discussed here. The second is for non-FDA approved manufacturer's methods and in-house developed assays, which will be discussed in a future SLI Newsletter. Verification of test performance requires more than simply quoting the characteristics described in the manufacturer's package insert or from a literature reference. Since variability between laboratories can influence test perfomance, each laboratory must demonstrate and document performance characteristics for each test method used.

CLIA regulation [§493.1213(b)][1] requires that laboratories using FDA approved methods have supporting documentation that their test results meet performance specifications comparable to those established by the manufacturer for accuracy, precision and the reportable test range.

To verify the manufacturer's reportable range, the laboratory can check the calibration by testing three or more solutions with known values as unknowns that expand the reportable range. For example, select patient samples that have previously been run using the old method, and which have values ranging within the manufacturer's reportable range. Then run these samples as "unknowns" using the new method. Plot the assigned values (values from the old method) on the x-axis and the observed values (values from the new method) on the y-axis. Note that zero cannot be used as a valid point. Review this data to determine if the new results fall within the established acceptability criteria for the laboratory.

In addition, the CLIA regulation under [§493.1213(b)(1)][1] states, "The laboratory must also verify that the manufacturer's reference range is appropriate for the laboratory's patient population."

[1] Code of Federal Regulations, Title 42, Vol. 3, Part 493, p. 881; Revised as of October 1, 1988; from the U.S. Government Printing Office via GPO Access [CITE: 42CFR493].

Program Reports —

Virus Isolation Laboratory 1998-1999 Influenza Summary

by Kristin Myers

The early diagnosis of influenza can limit the spread of illness and help reduce inappropriate use of antibiotics. Thus, rapid tests are an important diagnostic tool. The use of conventional viral culture remains critical, however, to isolate viruses which can be characterized to obtain information on subtypes and strains. Data on circulating strains aids in assessing current vaccine and in formulating vaccine for the following year. Two hundred eighty-eight specimens and isolates were submitted to the Virus Isolation Laboratory for testing and typing during the 1998-1999 influenza season. Of these, 62 were influenza isolates which had tested positive at various area hospitals and were sent for confirmation and sub-typing.

Of 72 positive primary specimens, sixty tested positive for influenza A-H3N2-"Sydney-like", seven were positive for influenza B-"Beijing-93-like", and two were positive for influenza A-H1N1 (see Table 1). In addition, although not subtyped, one specimen was identified as influenza A by the shell vial assay and two others by a rapid influenza A kit. Two throat swabs were positive for parainfluenza type 3. Another throat swab was HAD positive, not positive for parainfluenza but not able to be strain typed for influenza. This specimen was found to be influenza A-H3N2 by Restriction Fragment Length Polymorphism performed at the CDC. Of the isolates, 49 were identified as influenza A-H3N2, while 10 were influenza B-Beijing-93 (see Table 2). Two isolates were submitted from one 35 year-old patient. The first, collected March 1,1999 was typed as influenza A- H3N2, while the second, collected April 2, 1999, was influenza B-Beijing-93. Three of the 62 isolates did not grow in tissue culture at the State Laboratory Institute.

With respect to immunization status of the patients, seventy-four of those submitting specimens and one submitting an isolate were thought to have been vaccinated against influenza. Nonetheless, influenza A was isolated from 25 reportedly immunized patients and influenza B from three others. Table 3 indicates the breakdown of these cases by age. One hundred twenty-two of the patients submitting specimens were known not to have received vaccination this year, while the vaccination status of the remaining 91 patients is unknown.

The first positive primary specimen was identified as influenza B-Beijing from a throat swab collected on November 12, 1998. Subsequently, influenza A-H3N2 was isolated from a throat swab collected on November 30, 1998. Despite the appearance of positive specimens in November, additional influenza A specimens and isolates did not arrive until January, while additional specimens and isolates positive for influenza B were not collected until early February. Although influenza is a winter problem in Massachusetts, recent reports indicate current activity in Alaska and the Yukon Territory.

TABLE 1: DISTRIBUTION OF PRIMARY SPECIMENS BY AGE

PATIENT AGE (YEARS)	TOTAL SPECIMENS	INFLUENZA POSITIVE SPECIMENS	A-H3N2 POSITIVE	B-BEIJING POSITIVE	A-H1N1 POSITIVE	A NOT TYPED
<1	1	0	0	0	0	0
1-4	2	0	0	0	0	0
5-24	52	12	7	3	0	2
25-44	55	19	15	3	0	1
45-64	33	10	10	0	0	0
>65	83	31	28	1	2	0
TOTAL	226	72	60	7	2	3

TABLE 2: DISTRIBUTION OF ISOLATES BY AGE

PATIENT AGE (YEARS)	TOTAL ISOLATES	INFLUENZA POSITIVE ISOLATES	A-H3N2 POSITIVE	B-BEIJING POSITIVE	NO VIRAL GROWTH
<1	5	3	2	1	2
1-4	3	3	3	0	0
5-24	3	3	2	1	0
25-44	13	13*	10	3	0
45-66	15	14	13	1	1
>65	22	22	18	4	0
UNKNOWN	1	1	1	0	0
TOTAL	62	59	49	10	3

* Two separate isolates were submitted from specimens collected one month apart from one patient. The first was positive for influenza A-H3N2, while the second was positive for influenza B-Beijing-93. Each one is counted as a separate isolate.

TABLE 3: PATIENTS WITH REPORTED VACCINATION WHO TESTED POSITIVE FOR INFLUENZA VIRUS

PATIENT AGE YEARS	TOTAL PATIENTS	INFLUENZA A POSITIVE	INFLUENZA B POSITIVE
5-24	4	1	0
25-44	14	4	2
45-66	12	4	0
>65	45	16	1
TOTAL	75	25	3

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for the electronic version of this newsletter and other information about the State Laboratory Institute: www.state.ma.us/dph/bls.

Laboratory Training Activities

Response to Bioterrorism - Role of the Clinical Laboratory, State Laboratory Institute, Boston, MA: September 9, 1-day national conference, speakers from CDC, the FBI, Medical and Public Health Communities. Call (617) 983-6285.

Wet Mounts, State Laboratory Institute, Boston, MA: September 15 (PM), an STD/HIV Prevention Training Center of New England Wet-Workshop. No charge. Call (617) 983-6945.

Public Health Teleconference Series, State Laboratory Institute, Boston, MA: Influenza - October 19; *Chlamydia trachomatis* - November 16. Fee $25 per site per program. Call (617) 983-6285.

State Laboratory Training Coordinator, *Garry R. Greer, BS, (617) 983-6608, E-mail: garry.greer@state.ma.us.*
For a list of NLTN courses in your area sign on to the Web at http://www.cdc.gov/phppo/dls/nltn.htm.

The State Laboratory Institute Newsletter is a free monthly publication of the Bureau of Laboratory Sciences

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